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[54]发明名称 槲皮素衍生物及其医药用途

[57]摘要

本发明涉及槲皮素衍生物，及其制备方法，含有它的药物组合物及它们作为预 防或治疗与$5HT_{1A}$有关疾病，尤其是预防或治疗抑郁或焦虑的用途。

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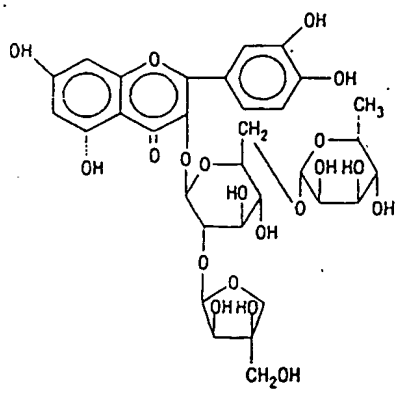
权 利 要 求 书

1. 式 I 化合物



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2. 含式 I 化合物及药用载体的药物组合物。

3. 具有预防或治疗与 $5HT_{1A}$ 有关疾病或症状的式 I 化合物或含式 I 化合物的药物组合物。

4. 根据权利要求 3 的化合物或药物组合物，其中所述与 $5HT_{1A}$ 有关疾病或症状是抑郁或焦虑。

说　　明　　书

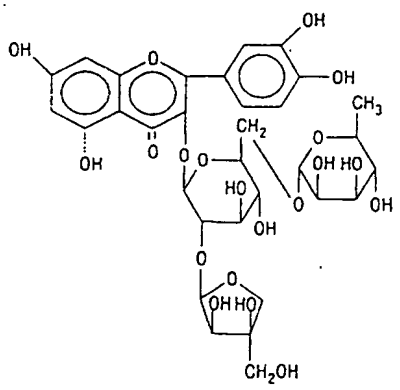
槲皮素衍生物及其医药用途

本发明涉及槲皮素衍生物，及其制备方法，含有它的药物组合物及它们作为预防或治疗与 $5HT_{1A}$ 有关疾病，尤其是预防或治疗抑郁或焦虑的用途。

属于棉花籽类的无毒棉花籽是无毒棉花成熟果实的种子，一般作为牲畜的饲料。无毒棉花籽是棉葵疼（Malvaceve）植物经过基因工程改造大一种新型农作物。到目前为止，尚未见到包括无毒棉花籽的棉花籽中槲皮素化学成分及其生物活性的报道。

本发明的目的，是寻找包括无毒棉花籽的棉花籽中具有生物活性的化学单体，并进而开发该化学单体的医药用途。

本发明者经过广泛深入的研究，现已从例如无毒棉花籽中提取出具有式 I 的槲皮素衍生物。结果发现，它可做为 $5HT_{1A}$ 受体的配基，并显示出良好的治疗和预防与 $5HT_{1A}$ 有关的疾病和症状，如：抗胃溃疡、抗十二指肠溃疡、对心脏和血压的调节以及对中枢系统的疾病，如抗抑郁、抗焦虑等的活性。本发明基于以上发现得以完成。



式 I 的结构式

本发明涉及式 I 槲皮素－3－O－α－D－芹菜糖－（1→2）－〔α－D－鼠李糖－（1→6）〕－β－D－葡萄糖甙，它可作为 $5HT_{1A}$ 受体的配基并且显示出良好的预防或治疗与 $5HT_{1A}$ 有关疾病，尤其预防或治疗

抑郁或焦虑的活性。

本发明还涉及药物组合物，其包括式 I 化合物及药用载体。

本发明还涉及作为预防或治疗与 $5HT_{1A}$ 有关的疾病或症状，尤其是预防或治疗抑郁或焦虑的式 I 化合物

本发明还涉及作为预防或治疗与 $5HT_{1A}$ 有关疾病或症状，尤其是预防或治疗抑郁和/或焦虑的含式 I 化合物的药物组合物。

根据本发明，本发明式 I 化合物或药物组合物可通过口服，非肠道或局部途径给药，给药剂型可以是例如片剂，胶囊，溶液，悬浮液，注射液、滴注液等。

根据本发明，本发明药物组合物可按本领域已知方法制备，例如将式 I 化合物与药用载体混合。

根据本发明，式 I 化合物：槲皮素－3－0－α－D－芹菜糖－（1→2）－〔α－D－鼠李糖－（1→6）〕－β－D－葡萄糖甙是从例如无毒棉花籽中得到的。所用的有机体溶剂包括醇类如甲醇、乙醇、丙醇、丁醇等；卤代烷类如二氯甲烷、三氯甲烷等；酯类如乙酸甲酯、乙酸乙酯、乙酸丙酯等；以及醚类如石油醚、乙醚。

下面的实施例及生物活性实验，是对本发明的进一步详细说明，但不意味着对本发明的任何限制。

实施例 1

式 I 槲皮素－3－0－α－D－芹菜糖－（1→2）－〔α－D－鼠李糖－（1→6）〕－β－D－葡萄糖甙的制备。

将无毒棉花籽 1kg 粉碎，然后过 100 目筛，筛过物用石油醚提取。每次用石油醚 5 升，提取 3 次。药渣用乙醇提取，每次用乙醇 8 升，提取 3 次，合并。减压蒸发至衡重。得到乙醇提取物 260g 乙醇提取物用水溶解，将其在在正丁醇/水中进行分析。得到正丁醇提取物 10g，得到水提取物 200g。将正丁醇取物上硅胶柱层析分离，展开剂是正丁醇：醋酸：水 = 7：1：2。得到式 I 化合物。

式 I 化合物为黄色粉末。10% EtOH-H_2SO_4 呈红黑色（加热），表明有糖存在。紫外 254nm 处有亮白色荧光，表明是黄酮类化合物。IR 光谱（KBr）：cm^{-1}3412(OH), 2925, 1654(C=O), 1608, 1361, 1201，表

明有羰基和羟基存在；UV 光谱（MeOH）：256.2(log ε 3.95), 354.6 (log ε 2.83)，为典型的黄酮醇类紫外特征。FAB－MS（m/z 743 $[M+H]^+$），确定其分子量是 742。化合物 I 核磁数据见表一。

表一：化合物 I 在 400MHz 中 ^1H－NMR 和 ^{13}C－NMR 数据

位置	δ_H(J Hz)	δ_C
2	----	156.52
3	----	132.81
4	----	177.04
5	----	161.23
6	6.12 s	98.81
7	----	166.30
8	6.30 s	93.86
9	----	156.10
10	----	103.28
1′	----	121.87
2′	7.57 dd(2.0, 1.6)	115.24
3′	----	145.01
4′	----	147.00
5′	6.82 dd(8.48, 1.6)	115.80
6′	7.72 dd(8.84, 2.0)	120.80
1g	5.53 d(7.6)	100.70
2g	3.52 d(7.6)	76.99
3g	----	76.88
4g	----	70.36
5g	----	75.69
6g	4.39 s	66.88
1r	3.11s	99.16
2r	----	70.30
3r	3.09 s	70.55
4r	----	71.84
5r	----	68.30
6r	5.39 s	17.78
1a	3.84 brs	108.64
2a	----	76.17
3a	3.86 s	79.33
4a	-----	74.01
5a		64.34

实施例 2

本发明式 I 化合物：槲皮素－3－O－α－D－芹菜糖－（1→2）－〔α－D－鼠李糖－（1→6）〕－β－D－葡萄糖甙的抗抑郁实验。

1.对大鼠大脑皮层腺苷酸环化酶活性的影响

（1）方法:

雄性 Wistar 大鼠，体重 200±20g，断头处死并分离大脑皮层，按文献方法 4℃提取突触膜（参见 Rasenick MM 等人，Pro Natl Acad Sci USA, 1980;77;4628）并用缓冲液悬浮，使蛋白浓度达 3～5mg/ml. 由于腺苷酸环化酶（adenylyl cyclase, AC）位于突触膜上，故需提前用药物与突触膜孵育，方法如下：含相应待试药物浓度的总体积为 100μl 的反应液中各成分终浓度为：15mmol. L^{-1}HEPES，PH7.5, 5mmol. $L^{-1}MgCl_2$, 1mmol. L^{-1}EGTA，1mmol. L^{-1}DTT，60mmol. L^{-1}NaCl，1mmol. L^{-1} 氨茶碱，0.5mg/ml 磷酸肌酸，0.14mg/ml 磷酸肌酸激酶，各反应管分别加入 20μg 的突触膜并立即置 30℃水浴中反应 10 分钟，此反应 20 分钟内是线性的，然后立即将反应管置于沸水中煮 3 分钟，终止反应。利用 cAMP 试剂盒（购自中国原子科学研究院）测量各反应管中 cAMP 产生量，测定过程全部在冰浴中进行，反应总体积 130μl，按试剂盒说明加入各种试剂，反应结束后，4000rpm 离心 7 分钟，吸取上清 120μl 置测量杯中，加 1.5ml 无水乙醇，摇匀后再加闪烁液 3.5ml，盖严、摇匀，过夜后在 Wallac 1409 液闪仪上测定样品 cpm 值，根据标准曲线及 cpm 值推算 cAMP 产生量。结果用 ANOVA 统计分析，组间比较 Dunnett's T 检验，结果见表二及表三。

表二.丙咪嗪及丁螺环酮对 AC 的激活效应

药物	cAMP 产生量(pmol/mg 蛋白/分钟)			
	25μM	100μM	400μM	1mM
丙咪嗪	15.07±4.91	18.53±3.42*	30.32±5.63***	79.79±21.38***
丁螺环酮	19.52±5.46*	19.71±5.57*	24.63±3.49***	33.00±8.58***
生理盐水	13.47±1.92			

表三. 式 I 化合物 AC 激活效应的比较

样品	cAMP 产生量(pmol/mg 蛋白/分钟)			
	13.5μM	40.5μM	135μM	405μM
式 I 化合物	23.27±4.95*	4.75±6.33***	43.42±4.78***	68.34±10.45***
盐水	13.47±1.92	------	----	------

X±SD vs 对照组, *P<0.0,5, **P<0.01, ***P<0.001

（2）讨论:

研究表明，抗抑郁剂对突触膜 AC 有急性激活效应，此效应可能是其作用机制的重要步骤，从表 1 看出，经典抗抑郁剂丙咪嗪及非典型抗抑郁剂丁螺环酮呈剂量依赖性地激活 AC. 式 I 化合物在 13.5μM（0.01mg/ml）时就显著激活 AC，达到 23.27±4.95 pmol/mg 蛋白/分钟，比丙咪嗪及丁螺环酮 25μM 时效应都强。式 I 化合物在 404μM（0.3mg/ml）效应达 68.34±10.45 pmol/mg 蛋白/分钟，比相同剂量下丙咪嗪以及丁螺环酮强 2～3 倍. 由此看出，式 I 化合物有抗抑郁作用，而且活性较强.

2.式 I 化合物对皮质酮损伤的 PC－12 细胞的保护作用

（1）方法:

用含 5% 小牛血清及 5% 马血清的 DMEM 培养液将 PC－12 细胞稀释为每毫升悬液含 2×10^5 个细胞，然后接种于预先用多聚赖氨酸处理过的 96 孔板中，于 37℃，5% CO_2 条件下培养 2～3 天，等细胞长满孔底后即可用于实验。吸去培养液，加入含相应药物浓度及 10^{-4}mol. L^{-1} 皮质酮的无血清 DMEM，48 小时后各孔加入 5mg/ml MTT10μl 并轻轻摇匀，4 小时后，每孔加 10% SDS100μl 并摇匀，置 37℃ 孵箱中过夜（8～12 小时），待深蓝结晶全部溶解后，轻轻摇匀，在酶标仪上测量各孔在 570nm 波长时的吸光度值（A），用 ANOVA 统计分析，结果见表四。

表四. 式 I 化合物对皮质酮损伤的 PC－12 细胞的保护作用

药物（μM）	吸光度（A）	A 值升高百分率（%）
正常对照	0.77±0.12	
损伤对照	0.24±0.04	
式 I 化合物		
4.04	0.74±0.14**	208.3
13.48	0.48±0.08***	250.0
40.43	0.86±0.10***	258.3
134.77	0.77±0.11***	220.8
404.31	0.61±0.16**	154.2

**P<0.01, ***P<0.001

（2）讨论

由表 4 中数据可以看到，式 I 化合物在浓度 4.04μM 时 A 值升高百分率达 208.3%，A 值升高百分率越大，表明对皮质酮损伤的 PC－12 细胞的保护作用越强。因此，本发明式 I 化合物对皮质酮损伤的 PC－12 细胞有很强的保护作用。

3.强迫游泳实验

（1）方法

按文献（Arch Int Pharmacodn Ther, 1977, 229(2):327）方法进行，小鼠 ip 给药物 30 分钟放入敞口玻璃缸内（高 19cm、直径 12cm），缸内水深 8cm，水温 22～23℃，小鼠置水中 6 分钟，用 Vidio 运动解析仪观察小鼠后 4 分钟内的累计不动时间及活动性，统计方法同前，结果见表五。

表五. 式 I 化合物对小鼠强迫游泳行为的影响

药物	不动时间(sec)
生理盐水	184.94±19.15
式 I 化合物 (mg/kg)	
0.31	148.69±30.81*
1.25	149.94±34.87*
5.00	134.38±40.99**

（2）讨论

小鼠强迫游泳行为实验是经典的抑郁模型，动物置于水中先是挣扎，企图逃脱，最后进入绝望状态而静止不动，抗抑郁剂能明显缩短不动时间，表5看出，本发明式I化合物低剂量0.31mg/kg即可使不动时间缩短，因此，化合物式I抗抑郁活性较强。

4.$5HT_{1A}$受体实验

（1）方法：按照表六的实验方法进行操作。

（2）讨化：$5HT_{1A}$受体实验表明，化合物式I与$5HT_{1A}$有较强的结合率。其IC_{50}在10^{-11}mol左右。

表六.$5HT_{1A}$受体实验

试液	反应体系及体积(μL)	总结合	非特异结合
标记配基	20nMOL H^3-OH-DPAT液	20	20
非标记配基	1mMOL 5-TH硫酸肌肝液	——	20
受体膜制剂	1：5大鼠脑皮层悬液	50	50
缓冲溶液	50mMOL Tris-HCl pH6.4缓冲液	130	110
上述溶液混匀后，在25℃的水浴中反应30分钟			

Certification

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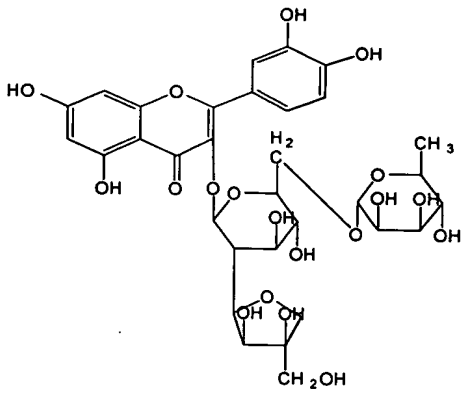
Claims

1. The compound of formula I



2. Pharmaceutical compositions containing the compound of formula I and pharmaceutical carriers.

3. The compound of formula I or pharmaceutical compositions containing the compound of formula I which are used for prevention or treatment of diseases or symptoms related to $5HT_{1A}$ receptor.

4. Compound or pharmaceutical compositions according to claim 3, wherein the diseases or symptoms related to $5HT_{1A}$ receptor are depression or anxiety.

Abstract

This invention relates to a quercetin derivative, its preparation method, and pharmaceutical combinations containing this compound, as well as their medical uses for the prevention or treatment of diseases related to $5HT_{1A}$ receptor, especially for the prevention or treatment of depression and anxiety.

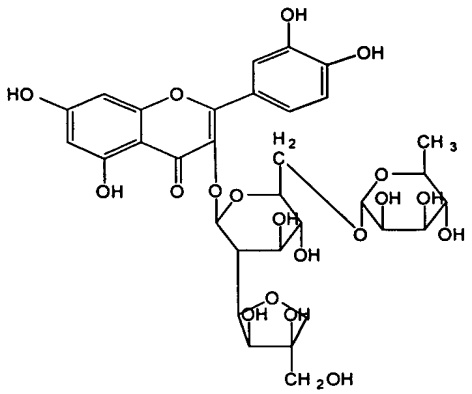
A Quercetin derivative and its medical usages

This invention relates to a quercetin derivative, the method of its preparation, and medicinal compositions containing this compound, as well as medical uses thereof for the prevention or treatment of diseases related to $5HT_{1A}$ receptor, especially for the prevention or treatment of depression and anxiety.

Glandless cottonseed, a variety of cottonseed, is the seed of glandless cotton, and normally used as feedstuff of livestock. Glandless cotton is a novel crop produced by selective breeding of cotton plant, a plant of the family Malvaceae. Up to now, the quercetin derivatives contained in cottonseeds (including glandless cottonseeds) and their biological activities have not been reported.

The purpose of this invention is to find out the biologically active chemical entities contained in cottonseed (including glandless cottonseeds), and to develop these chemical entities for medicinal uses.

Through extensive and intensive studies, the inventors have isolated the quercetin derivative shown in formula I. It was found that this compound could serve as a ligand of the $5HT_{1A}$ receptor. It exhibited favorable effects in treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, such as the effects of preventing gastric and duodenal ulcer, modulating heart function and blood pressure, as well as the effects on central neural disease such as depression and anxiety. This invention is achieved on the base of above discoveries.



Formula I

This invention relates to quercetin-3-O- α -D- apiosyl-(1 → 2)-[α -D-rhamnosyl-(1→6)]- β -D- glucoside, the compound shown in formula I. It can serve as a ligand of the $5HT_{1A}$ receptor, and has favorable effects in treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, especially in treating and preventing depression and anxiety.

The present invention further relates to pharmaceutical compositions comprising compound of formula I and pharmaceutical carriers.

The present invention further relates to the compound of formula I, for treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, especially for treating and preventing depression and anxiety.

The present invention further relates to pharmaceutical compositions containing the compound of formula I, for treating and preventing diseases and symptoms related to the $5HT_{1A}$ receptor, especially for treating and preventing depression and anxiety.

According to the present invention, compound of formula I and pharmaceutical compositions thereof, of the present invention, can be administered orally, parenterally or tropically. The dosage form may be, for example, tablets, capsules, solutions, suspensions, injections and intravenous dripping solutions, etc.

According to the present invention, the pharmaceutical compositions of

the invention can be prepared according to known methods in the art, for example, by mixing the compound of formula I with pharmaceutical carriers.

According to the present invention, quercetin-3-O- α -D- apiosyl-(1→2)-[α -D-rhamnosyl-(1→6)]- β -D-glucoside of formula I is obtained, for example, from glandless cottonseeds. The organic solvents employed include alcohols, such as methanol, ethanol, propanol, butanol; halogenated hydrocarbons such as methylene dichloride, chloroform; esters such as methyl acetate, ethyl acetate, propyl acetate; and ethers such as petroleum ether, ethyl ether.

The following examples and bioactivity experiments further illustrate the present invention and are not intended to limit the invention in any way.

Example 1

Preparation of quercetin-3-O- α -D-apiosyl-(1→2)-[α -D-rhamnosyl-(1→6)]- β -D- glucoside

1kg of glandless cottonseeds are crushed and then passed through a 100 mesh, followed by 3 times of extraction with 5L petroleum ether for each time. The residues are then extracted 3 times with ethanol, 8L for each time. The extraction solution is then merged and evaporated under reduced pressure to constant weight. The 260 g of ethanol extract obtained is then dissolved in water, and distributed in n-butanol/H_2O solution, to give 10g of n-butanol extract and 200g of water extract. The n-butanol extract is separated through a silica gel column to give the compound of formula I, the developing agent is n-butanol:acetic acid:H_2O=7:1:2.

The compound of formula I thus obtained is yellow powder and turns into dark-red when heated in 10% EtOH-H_2SO_4, which indicates the existence of saccharide. Bright white fluorescence observed at 254nm, indicates itself being a flavonoid. Absorption peaks in IR spectrum (KBr) of said compound, of 3412cm^{-1}(ν_{-OH}), 2925 cm^{-1}, 1654 cm^{-1}($\nu_{-C=O}$), 1608 cm^{-1}, 1361 cm^{-1}, 1202 cm^{-1}, indicate existence of carbonyl and hydroxyl; the UV spectrum of said

compound: 256.2nm (log ε 3.95), 354.6nm (log ε 2.83), shows typical spectrum of a flavonol. The molecule weight of compound of formula I is determined to be 742 by FAB-MS. NMR data of compound of formula I is shown in table 1.

Table 1: ^{1}H-NMR and ^{13}C-NMR data of compound of formula I (400MHz)

Position	δ_H (J Hz)	δ_C
2		156.52
3		132.81
4		177.04
5		161.23
6	6.12 s	98.81
7		166.30
8	6.30 s	93.86
9		156.10
10		103.28
1'		121.87
2'	7.57 dd(2.0, 1.6)	115.24
3'		145.01
4'		147.00
5'	6.82 dd (8.48, 1.6)	115.80
6'	7.72 dd(8.48, 2.0)	120.80
1g	5.53 d(7.6)	100.70
2g	3.52 d(7.6)	76.99
3g		76.88
4g		70.36
5g		75.69
6g		66.88
1r	4.39 s	99.16
2r	3.11 s	70.30
3r		70.55
4r	3.09 s	71.84
5r		68.30
6r		17.78
1a	5.39 s	108.64
2a	3.84 br s	76.17
3a	---	79.33
4a	3.86 s	74.01
5a		64.34

Example 2

Experiments on the anti-depression activity of compound of formula I: quercetin-3-O- α -D-apiosyl-(1 → 2)-[α -D-rhamnosyl-(1 → 6)]- β -D-glucoside

1 Effect on the activities of adenylyl cyclase (AC) in rat cerebral cortex:

(1) Methods

Male Wistar rats weighed 200±20g are sacrificed by decollation, and cerebral cortex is separated. Synaptolemma is extracted at 4℃ according to the method as described in literature (Rasenick MM et al, Pro. Natl. Acad. Sci. USA, 1980; 77:4628) and suspended in buffer solution, so that a protein concentration of 3～5mg/ml is reached. The synaptolemma has to be incubated in advance with the test medicine, as adenylyl cyclase (AC) is located on it. The experiment is carried out as follows: portions of 100μl reaction solution containing certain concentration of test medicine and 15mmol/L HEPES, pH=7.5, 5mmol/L $MgCl_2$, 1mmol/L EGTA, 1mol/L DTT, 60mmol/L NaCl, 1mmol/L aminophylline, 0.5mg/ml phosphocreatine and 0.14mg/ml phosphocreatine kinase are respectively dispensed into reaction tubes, followed by addition of 20μg synaptolemma to each tube. Then, the tubes are immediately put into a water-bath at 30℃ to react for 10 minutes, which reaction is linear during the first 20 minutes. After that, all the reaction tubes are immediately transferred into boiling water and deposited for 3 minutes to terminate the reaction. The amount of cAMP thus produced is measured in an ice-bath environment with cAMP kit, the total reaction volume is 130μL. The measure is carried out according to the instruction of the kit: various reagents are added and, after the reaction finishes, the tubes are centrifugated at 4000 rpm for 7 minutes. 120μl supernatant is then pipetted into the measure cup, added afterwards with 1.5ml anhydrous alcohol, after shaken up, 3.5ml of scintillation solution is added. Then, the cups are sealed and shaken up, and are left overnight. The values of CMP of all samples are determined then by Wallac 1409 liquid scintillation counter. The amount of

cAMP produced can be calculated according to the standard curve and cpm value. The results are statistically analyzed by ANOVA, and Dunnett's T test is made for inter-group comparision. The results are shown in table 2 and table 3.

Table 2 Activation effects of imipramine and buspirone on AC

Medicines	Amount of cAMP produced (pmol/mg protein/minute)			
	25 μ M	100 μ M	400 μ M	1mM
imipramine	15.07±4.91	18.53±3.2*	30.32±5.63***	79.79±21.38***
buspirone	19.52±5.46*	19.71±5.57*	24.63±3.49***	33.00±8.58***
physiological saline	13.47±1.92	---	---	---

Table 3 Activation effect of compound of formula I on AC

Medicines	Amount of cAMP produced (pmol/mg protein/minute)			
	13.5 μ M	40.5 μ M	135 μ M	405 μ M
Compound of formula I	23.27±4.95*	4.75±6.33***	43.42±4.78***	68.34±10.45***
physiological saline	13.47±1.92	---	---	---

X±SD vs control group, *P<0.05, **P<0.01, ***P<0.001

(2) Discussion

It is indicated that anti-depression agent has an acute activation effect on synaptolemma AC, which might be an important step of its mechanism. It can be drawn from table 2 that typical anti-depression agent imipramine and atypical anti-depression agent buspirone dose-dependently activate AC. The compound of formula I remarkably activates AC, under a concentration of only 13.5 μ M (0.01mg/ml), up to 23.27±4.95 pmol/mg protein/minute. This effect is stronger than those of 25 μ M imipramine and buspirone. The activation effect of said compound at 404 μ M (0.3mg/ml) amounts up to 68.34±10.45 pmol/mg protein/minute, 2~3 times higher than same doses of imipramine and buspirone. Therefore, it can be concluded that compound of formula I has

an anti-depression effect with relatively higher activity.

2. Protection effect of compound of formula I on PC-12 cells damaged by corticosterone.

(1) Methods

PC-12 cells are diluted into a suspension (2×10^5 cells/ml) with DMEM culture solution containing 5% calf serum and 5% horse serum, and then are transplanted into 96-well plates pretreated with polylysine, and cultivated under conditions of 37℃ and 5% CO_2 for 2～3 days. Cells are to grow all over the wells bottom before test. The culture solution is then pipetted away and serum-free DMEM is added containing certain concentration of test medicine and 10^{-4} mol/L corticosterone, 10 μl of 5mg/ml MTT is added 48 hours later to each well, shaking up slightly and, 4 hours later, 100 μl of 10% SDS is added to each well, again shaking up slightly. The plates are then left in the incubator overnight at 37 ℃(about 8～12 hours). After all the dark-blue crystals are dissolved, shaking up slightly and absorbance (A) of each sample at 570nm is read using microplate reader. The results are then statistically analyzed by ANOVA, and shown in table 4.

Table 4 Protection effect of compound of formula I on PC-12 cells damaged by corticosterone

Medicines (μ mol.L^{-1})	Absorbance (A)	Increase of A (%)
Normal control	0.77±0.12	
Damaged control	0.24±0.04	
Compound of formula I		
4.04	0.74±0.14**	208.3
14.38	0. 48±0.08***	250.0
40.43	0.86±0.10***	258.3
134.77	0.77±0.11***	220.8
404.31	0.61±0.16**	154.2

**P<0.01, ***P<0.001

(2) Discussion

Data in table 4 shows that the increase of A (%) of compound of formula I reaches as high as 208.3%, at a concentration of 4.04 μ mol/L. The higher the increase of A, the stronger the protection effect of said compound to PC-12 cells damaged by corticosterone. Therefore, said compound has a strong protection effect on PC-12 cells (rat pheochromocytoma cell strain) damaged by corticosterone, which is identical with the effect thereof on primary cultured hippocampal cells.

3 Forced swimming test

(1) Method

The test is carried out according to literature (Arch Int. Pharmacodn. Ther, 1977, 229(2): 327). 30 minutes after abdominal injection, the mice are put into an open glass box (19 cm high and 12 cm of diameter). Water inside the glass box is 8cm in depth and 22～23℃ in temperature. The mice are put into the water for 6 minutes and observed by Vidio movement analyzer, the accumulated immobility time of the mice during the last 4 minutes and their motility are statistically analyzed identically as above. The results are shown in table 5.

Table 5 Effect of compound of formula I on forced swimming behavior of mice

Medicines	Duration of immobility (sec)
physiological saline	184.94±19.15
Compound of formula I (mg/kg)	
0.31	148.69±30.81*
1.25	149.94±34.87*
5.00	134.38±40.99**

(2) Discussion

The forced swimming behavior test on mouse is a classical anti-depression model test. When the animal have been put in the water, it struggles and tries to escape at first, then enters a state of despair and becomes still and immobile. Anti-depressant can shorten the immobility time of the tested animal. Table 5 shows that compound of formula I can shorten the immobility time of mice at a dosage of 0.31mg/kg, therefore, the said compound has relatively high anti-depression activity.

4 $5HT_{1A}$ receptor test

(1) Method : The tests were carried out according to table 6.

(2) Result: The $5HT_{1A}$ receptor tests indicated that the compound of formula I had evident binding activity to $5HT_1$ receptor, the IC50 value was about 10^{-11}mol.

Table 6 the assay condition of $5HT_{1A}$ receptor test

reagents	components of the assay solutions	total binding test (μL)	non-specific binding or competition test (μL)
labeled ligand	20nM ^{3}H-8-OH-DPAT	20	20
non-labeled ligand or tested samples	1mM 5-HT creatinine sulfate or the tested sample in different concentration	--	20
receptor membrane	1 : 5 suspension of rat hippocampal membrane	50	50
buffer	50mM Tris-HCl pH6.4	130	110
the above-mentioned assay solutions is mixed and then incubated in a water-bath at 25℃ for 30 minutes.			